

STUDIES ON THE ANTISTREPTOMYCIN ACTIVITY OF
BRAIN INFUSION AND THE CHANGES IN
MORPHOLOGY OF BACTERIA SUBJECTED
TO STREPTOMYCIN AND THE
ANTISTREPTOMYCIN
FACTOR

BY

REYNOLD BERNARD CZARNECKI

B.S., Pennsylvania State College, 1939

M.S., University of Illinois, 1942

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1948

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The high quality of the photo-micrographs is typical of the work produced by Mr. Raymond R. Hamm, the manager of the University Photography Department.



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Research by Wallace *et al.* (1945) had shown that the effect of streptomycin *in vitro* on bacteria was dependent to a considerable extent on the type of medium in which the sensitivity was determined. Both *Eberthella typhosa* (Hopkin's strain) and *Staphylococcus aureus* (FDA 209) used in their study were able to grow in brain heart infusion in the presence of concentrations of streptomycin which proved harmful to these bacteria when a less nutritive medium was used. By fractionation experiments, Rhymer and Wallace (1947) were able to demonstrate that the brain infusion portion of the brain heart infusion broth was responsible for the antistreptomycin activity. A similar result was demonstrated by using Wolf's (1945) casamino acids medium to which was added phytone, a plant protein. Rhymer *et al.* (1947) found upon continued research that the chemical behavior of the antistreptomycin fraction which they had studied indicated that lipositol may be a substance capable of exerting this neutralizing activity. Lipositol was the name given by Woolley (1943) to an inositol-containing fraction of soy bean phosphatides. Rhymer *et al.* (1947) obtained some lipositol from Woolley and were able to show that it was capable of neutralizing or inactivating the streptomycin.

There had also been reported in the literature on this subject that streptomycin was capable of influencing the morphology of some bacteria. Schatz and Waksman (1944) reported elongation of cells of *Mycobacterium tuberculosis* due

to the presence of the compound. Strauss (1947) found that of the bacteria he subjected to streptomycin action, *Shigella sonnei* was most affected. Elongation, swelling and irregular staining by the Gram method were demonstrated by the cells of this species. Stubblefield (1947) isolated a variant of *Escherichia coli* and found that this organism developed into bizarre rods varying in size when exposed to streptomycin.

The experiments in the first part of the present study were carried out to determine whether a number of organisms other than *E. typhosa* (Hopkin's strain) and *S. aureus* (FDA 209) were able to grow in the presence of inhibitory quantities of streptomycin when brain infusion was added to a basal medium. The second part of the study was conducted to observe effects on morphology of various bacteria when streptomycin was present not only in graded quantities of strength but also when tests were carried out in three different media. These media were brain heart infusion, nutrient broth and Wolf's casamino acids medium.

EXPERIMENTAL

The studies on the antistreptomycin effect of brain infusion were carried out by first determining the quantity of streptomycin which prevented the bacteria from growing in Wolf's medium. This was a reproducible substrate. Some of the species grew poorly in this medium alone; hence a yeast supplement had to be added in these cases. As soon as inhibitory limits of the antibiotic were established, the bacteria were subjected to these inhibitory concentrations in their respective media together with increasing amounts of brain infusion. After 24 hours incubation at 37°C., the tubes were examined. Growth was determined by the presence or absence of turbidity. A table was compiled which effectively illustrated the results.

Morphology studies were conducted by subjecting *Bacillus subtilis*, *S. aureus* (FDA 209), *E. coli* (Stubblefield strain)

and *E. typhosa* (Hopkin's strain) to slight, moderate and strongly bacteriostatic concentrations of streptomycin in brain heart infusion broth, nutrient broth and Wolf's medium. Preliminary sensitivity determinations were made in order to determine what constituted slight, moderate and strongly bacteriostatic concentrations in each of the three media. At regular intervals of one, three, 12 and 24 hours, five milliliter samples were removed from each bottle containing the medium, the organisms and the antibiotic. In the case of *E. coli* (Stubblefield strain) in brain heart infusion broth, samples were removed so as to obtain a single one-hour exposure and also exposures at three-hour intervals throughout the entire 24-hour period. Each sample was centrifuged at high speed. The sediments were then spread on glass slides. They were stained with Loeffler's alkaline methylene blue and examined under oil immersion. *E. coli* (Stubblefield strain) was the only organism which showed changes in morphology which could be considered attributable to the streptomycin. As a result, photo-micrographs of glass slides and electron microscope photographs of unstained sediments on special screens were made only with this species.

Proper controls were used in both parts of the study.

DISCUSSION AND SUMMARY

The antistreptomycin effect of brain infusion was demonstrated with a number of bacteria in addition to *E. typhosa* (Hopkin's strain) and *S. aureus* (FDA 209). The amount of growth obtained was found to be related to the quantity of brain infusion present in the basal medium.

Streptomycin was found to exert a marked influence on the morphology of *E. coli* (Stubblefield strain). In brain heart infusion broth, the cells of this species become elongated and formed a variety of V, Y and comma shapes decidedly unlike the coccoid or cocco-bacillary shapes present in the original inoculum. This alteration in morphology was observed in slight, moderate and strong concentrations of the antibiotic.

In the slight and moderate concentrations, there was a tendency for the cells to revert to normal form after continued incubation. The cells in the strong concentration retained their abnormal shapes throughout the entire 24-hour period. In nutrient broth and Wolf's medium, only elongation of cells occurred.

B. subtilis, *S. aureus* (FDA 209), *E. coli* 251 and *E. typhosa* (Hopkin's strain), on the other hand, did not exhibit changes in cell morphology as a result of exposure to streptomycin in any of the three media studied.

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VITA

The author was born October 28, 1913 in Erie, Pennsylvania. He attended parochial and public schools in that city and graduated from Erie East High School in 1931. Following graduation, he attended the Junior College of the University of Pittsburgh in Erie, Pennsylvania part and full time until 1935. A transfer was then made to the Pennsylvania State College where he received a Bachelor of Science degree in bacteriology in 1939. One year of graduate study from 1939-1940 then followed at the University of Wisconsin. This was succeeded by a year's service in the Post Office at Erie, Pennsylvania. A research assistantship was received in the Department of Home Economics of the University of Illinois and after a year of study, the degree of Master of Science in bacteriology was conferred in 1942. This was followed by enlistment in the Army of the United States which began three and a half years of military service in various army hospital laboratories and a medical general laboratory. Upon separation from the service in 1946, the author returned to the University of Illinois to begin studies leading to a Doctor of Philosophy degree in bacteriology.

He is a member of Pi Tau Phi, Gamma Sigma Delta, Phi Kappa Phi, Phi Beta Kappa, Phi Sigma, Sigma Xi, the Society of American Bacteriologists and the American Association for the Advancement of Science.

